REMARKS

Status of the Claims

Claims 1 – 9, 12 – 17, and 19 – 37 are currently pending. No claims are presently amended.

Rejection of Claims 1 – 9, 12 – 17, and 19 – 37 under 35 USC 102(b) over EP 0 521 562 ('DeVringer')

Claims 1 – 9, 12 – 17, and 19 – 37 stand rejected under 35 USC 102(b) as allegedly being anticipated by EP 0 521 562 ('DeVringer'). The Applicants respectfully traverse.

The instantly rejected claims broadly concern powders of reversed vesicles.

The Office alleged in its latest Office Action that "Although ***EP does not explicitly teach that the preparation is in the form of a powder***, since it teaches on col. 12, lines 55-56, the removal of the non-polar vehicle (volatile silicon oil), ***the teachings of a powder form of the preparation are implicit in the reference***." (Emphasis added.)

The applicants previously argued and continue to argue that although DeVringer instructs to "remove the non-polar excipient(s) to obtain an instant preparation," DeVringer fails to teach a method for doing so. Without a teaching of how to remove the non-polar vehicle, the ordinary artisan could not arrive at the presently claimed powder of reversed vesicles.

In order to sustain an anticipation rejection based on inherency, the inherent element must necessarily be present in the prior art, and, furthermore, it is incumbent on the Office to provide evidence or scientific reasoning demonstrating the allegedly inherent feature is necessarily present:

> **The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic.** *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (reversed rejection because inherency was based on what would result due to optimization of conditions, not what was necessarily present in the prior art); *In re Oelrich*, 666 F.2d 578, 581-82, 212 USPQ 323, 326 (CCPA 1981). "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. **Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.**'" *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999) (citations omitted) [...] >Also, "[a]n invitation to investigate is not an inherent disclosure" where a prior art reference "discloses no more than a broad genus of potential applications of its

> discoveries." *Metabolite Labs., Inc. v. Lab. Corp. of Am. Holdings*, 370 F.3d 1354, 1367, 71 USPQ2d 1081, 1091 (Fed. Cir. 2004) [...]
>
> **"In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art."** *Ex parte Levy,* 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990) (emphasis in original) ...

MPEP 2112 (IV) (emphasis added).

The applicants maintain that the Office has not provided evidence or scientifically based reasoning that as of the time of filing the present invention one of ordinary skill in the art would have necessarily known of and employed a method of removing non-polar vehicles that would necessarily lead not merely to a powder, but to a powder of reversed vesicles having the properties recited in the present claims. Without such a showing, this rejection cannot stand.

Ex parte Levy (cited *supra*) is particularly relevant to the instant situation. In *Ex parte Levy*, the disputed claims had been rejected over a reference cited to inherently disclose the claimed product. However, the rejection was reversed by the Board of Patent Appeals and Interferences. Therein, the reference was found to be lacking in that it, (*ibid.* 1464; emphasis in original)

> ...does not provide any working example revealing the process conditions employed to produce the catheter balloon. We have *only* a general invitation to employ "injection blow molding."

In the instant case, as in *Ex parte Levy,* there is only a teaching to do something but not of how to do it in a way that yields the claimed product.

It cannot be assumed that methods which may be viable for producing powders of vesicles, micelles, or reverse micelles would be applicable to producing the presently claimed powder of reverse vesicles due to the non-trivial differences in composition and structure of each of the preceding with respect to **reverse vesicles** *(vide infra)*. Importantly, not every method of removing the non-polar vehicle from the various dispersions of DeVringer will result in a powder as presently claimed. That is, a powder as presently claimed would not necessarily result from removing the non-polar vehicle in the preparations of DeVringer.

Furthermore, the applicants note that DeVringer merely recites a process that includes removing the non-polar vehicle from a dispersion of vesicles or polymerized vesicles to obtain an instant preparation. DeVringer does not disclose the instant preparation itself.

Finally, in the rejection under 35 U.S.C. § 103 of the present (and previous) Office Action the Office has stated that DeVringer does not teach the presently claimed powder. The Office cannot have it both ways; it cannot both assert that DeVringer both discloses the presently claimed powder and therefore anticipates the claims and at the same time allege that DeVringer does not disclose the presently claimed powder but that with additional art renders the powder obvious. On this basis alone at least one of these rejections must be withdrawn.

In conclusion, each element of the instantly rejected claims is not disclosed by DeVringer, as required for a rejection under 35 USC 102(b). The instant rejection of claims 1 – 9, 12 – 17, and 19 – 37 under 35 USC 102(b) is improper, and the Applicants respectfully request its reconsideration and withdrawal.

Rejection of Claims 1, 8 – 9, 13 – 16, 20, 27, 28, and 35 – 37 under 35 USC 102(e) over US Pat. No. 5,693,516 ('Blinkovsky')

Claims 1, 8 – 9, 13 – 16, 20, 27, 28, and 35 – 37 stand rejected under 35 USC 102(e) as allegedly being anticipated by US Pat. No. 5,693,516 ('Blinkovsky'). The Applicants respectfully traverse.

Blinkovsky relates to methods for preparing a protein composition which is soluble in an organic solvent, the method comprising preparing a ***reverse micelle*** **solution** from a surfactant, a protein in aqueous solution, and a water immiscible organic solvent, and evaporating the solution to dryness and the product obtained according to this process (see Abstract; and Column 1, ll. 47 - 52). The instantly rejected claims concern ***reversed vesicles*** **powder**, which is a distinctly different system.

One skilled in the art would readily recognize that micelles and vesicles, and the reversed forms of each, are fundamentally different entities. A "micelle" is an aggregate formed by a surfactant in solution such that, when formed in a polar environment (*e.g.*, water), the non-polar portion of the surfactant is in the interior of the aggregate and the polar portion is on the exterior. Thereby, the interior of the aggregate is non-polar, while the exterior is polar. For a reversed micelle, the interior of the aggregate is polar, while the exterior is non-polar.

In contrast, a "vesicle" is an aggregate formed by surfactants, which form a bilayer structure where each side of the bilayer contains the polar portion of the surfactant and the non-polar portions of the surfactant are on the interior of the bilayer; a vesicle is merely a closed container (in simplest terms, a sphere) formed by such a bilayer. When formed in a polar

environment (*e.g.*, water), both the interior and exterior of a vesicle are polar. For a reversed vesicle, the interior of the bilayer forming the vesicle is polar, while each exterior side of the bilayer is non-polar; it follows that both the interior and exterior of a reversed vesicle are non-polar.

Such definitions for micelles and vesicles are familiar to those skilled in the art; for example see page 80 of Kunieda *et al.,* "Formation and Structure of Reverse Vesicles," in Rosoff, ed. *Vesicles*, Marcel Dekker: New York, 1996, pp. 79-103 ("Kunieda"). For the convenience of the Office, a copy of Kunieda is included with this response. We note, on page 80 under "I. Introduction" in the first and second paragraphs, it is described that surfactant molecules in a solvent can aggregate to form a variety of structures, including micelles, reverse micelles, and bilayers. The size and shape of these self-organizing structures in ternary water/surfactant/oil systems is dictated by such parameters as temperature, pressure, concentration and the interactions between surfactant, water and oil molecules. Figure 2 on page 83 shows a phase diagram illustrating the different physical systems observed at different temperatures dependent on the concentration of oil/water; Figure 3 on page 85 show phase diagrams illustrating the different physical systems observed dependent on the concentration of the component water/surfactant/oil. Explanatory notes can be found for Figure 2 on page 82 (starting in second paragraph under "II. Surfactant phase behavior and self-organizing structures") and for Figure 3 on page 84 (starting in second full paragraph).

Further, Blinkovsky provides its own definition for the term "reverse micelles solution" (Column 2, ll. 1 - 4): a water-in-oil microemulsion comprising droplets having a size of between 0.0015-0.2 μm. Blinkovsky distinguished a reverse micelle solution from a "reverse phase emulsion," which is a water-in-oil emulsion which has a droplet size of 0.2-100 μm (Column 2, ll. 5-9). According to Blinkovsky, these two categories are also distinguished by appearance (turbid for a reverse phase emulsion, transparent for a reverse micelle solution), and thermodynamic stability (unstable for a reverse phase emulsion, stable for a reverse micelle solution). A reverse phase emulsion will not provide the same results as a reverse micelle solution, as shown in the examples (Column 2, ll. 9-15).

In view of Blinkovsky's own definitions, it is also clear that a reversed micelles solution is distinct from the reversed vesicles dispersion product according to the present invention. The dispersion of reversed vesicles of the instant claims, as assessed with polarized light microscopy,

exhibit Maltese crosses (as a parameter for vesicles) and the particle size of the vesicles ranged from 1 - 16 μm (Example 6 in connection with 5) to 120 - 5138 μm (Example 7 in connection with 5).

Finally, in the method as described by Blinkovsky, it is required that a reverse micelle system be formed by a protein, an organic solvent, water and a surfactant. According to the instant claims, reversed vesicle dispersions are prepared by mixing a surfactant, a non-polar phase and optionally a lipophilic and/or hydrophilic stabilizing factor and bio-active agent. The product obtained by evaporating the transparent solution representing the reverse micelles system according to Blinkovsky ***is a soap-like solid or viscous liquid*** (Column 3, ll. 26-27), and therefore ***not a powder***, which is the product obtained by the process according to the instant claims.

The state of the art at the time of filing of the instant application with respect to reverse vesicles is reflected by the enclosed Kunieda reference. On page 80, third paragraph, Kunieda states that until recently [1996], little attention had been paid to reverse vesicles. It is interesting to note that Kunieda describes various reverse vesicular systems (starting at page 87 under B.), but notes that only few combinations resulting in reverse vesicular systems had been investigated (paragraph bridging pages 88 and 89). In particular, under "IV. Reverse vesicles in a sucrose monoalkanoate system," reverse vesicular systems in an alkane or a combination thereof with an alkanol are extensively described. Under "V. Methods to produce reverse vesicles," two methods are described to obtain a dispersion of reverse vesicles in decane.

From Kunieda, it becomes clear that (in 1996, at least) reverse vesicular systems were a developing field of research. Kunieda's failure to mention either reverse vesicles in a biodegradable oil or the preparation of powders comprising reverse vesicles, suggests that little or nothing of importance had been published regarding these systems; such evidence indicates that the presently claimed powders and methods for obtaining dispersions of reverse vesicles in biodegradable oils were not yet contemplated. The teachings of Blinkovsky, both with respect to a manufacturing method for ***reverse micelles*** and for the product obtained after removing the external phase does not anticipate the instant claims directed to ***reversed vesicles***. The Applicants submit that the instant rejection of claims 1, 8 – 9, 13 – 16, 20, 27, 28, and 35 – 37 under 35 USC 102(e) is improper and respectfully request its reconsideration and withdrawal.

Rejection of Claims 1 – 9, 12 – 17, and 19 – 37 under 35 USC 103(a) over EP 0 521 562 ('DeVringer') in light of one or more of EP 0 678 295 ('Citernesi'), EP 0 159 237 ('Lafon'), GB 2,002,319 ('Schneider'), and JP 05194253 ('Hiroshi')

Claims 1 – 9, 12 – 17, and 19 – 37 stand rejected under 35 USC 103(a) as allegedly being obvious by combining the teachings of EP 0 521 562 ('DeVringer') and one or more of EP 0 678 295 ('Citernesi'), EP 0 159 237 ('Lafon'), GB 2,002,319 ('Schneider'), and JP 05194253 ('Hiroshi'). The Applicants respectfully disagree.

In making the present rejection, the Office has conceded that DeVringer lacks a teaching of a powdered preparation,[1] and has cited each of the noted documents to allegedly cure the admitted deficiencies of DeVringer. In view of the long lists of components proposed in DeVringer to be used for the non-polar phase, surfactant, and lipophilic and hydrophilic stabilizing agents, there can be no uniform measure or method to remove all possible non-polar excipients from dispersions of reversed vesicles.

In DeVringer (at Column 12, ll. 55-56) removal of the non-polar excipient was merely mentioned as a possible further production step. DeVringer does not specify any enabling method to remove the excipient, any result of such a removal (*i.e.*, no examples), and/or any advantage or motivation why such production step should be taken, let alone whether or not this step would be feasible.

Starting with the teachings of DeVringer, a certain sequence of thoughts and ideas would be required to obtain the powder of the instant claims consisting of reversed vesicles comprising one or more non-ionic surfactants. Such a sequence can be described by the following steps:

- selecting a combination of a non-ionic surfactant and a non-polar excipient to prepare a dispersion of reversed vesicles;
- selecting methods in the art to remove an external phase from a multi-phase system;
- selecting the conditions under which such methods could be made applicable to the above-mentioned dispersion of reversed vesicles;
- applying the method to the dispersion of reversed vesicles; and
- verifying that the removal of the external phase did not result in changes in the structure of the vesicle.

[1] As discussed with regard to the 35 U.S.C. § 102 rejection above, if the Office takes the position set forth in this § 103 rejection, it necessarily must withdraw the § 102 rejection.

None of the cited documents, alone or in combination, would have provided the person of ordinary skill in the art any teachings to bridge the gap between the dispersions of reversed vesicles in DeVringer and the powdered form of the instant claims.

Citernesi and Schneider, although related to vesicular dispersion preparations, each contain a polar external phase and not a non-polar phase.

Citernesi relates to a completely different issue than DeVringer and the instant claims. Citernesi is concerned with how to load an active principal into liposomes. It is not concerned with increasing the amount of the liposome vehicle, let alone reversed vesicles in a non-polar vehicle. Citernesi relates to providing a method to manufacture liposomes (vesicles in a polar vehicle) exhibiting a high drug content by the formation of a drug-phospholipid complex in an organic solvent first, removing the organic solvent, and then adding the polar vehicle to the residue thus obtained.

Schneider, aims at providing a solution for the short shelf life of liposomes (vesicles in a polar vehicle) by providing a process for the dehydration of the liposomes – by lyophilization under the addition of a hydrophilic compound - to obtain a powder which can be stored for a longer period and from which, and with an aqueous medium, a liposome dispersion can be re-constituted. However, during lyophilization about 30% of the liposomes were destroyed and therefore there is a decrease in percent yield of liposomes. As Schneider deals with liposomes in a polar medium (rather than reversed vesicles in a non-polar medium) and teachings a loss of 30% of the liposomes, one of ordinary skill in the art seeking to improve the percent yield of (reversed) vesicles in a non-polar vehicle would not be inclined to apply the teachings of this document to this problem.

As illustrated by the both Schneider and Citernesi, these techniques for removing an external vehicle are commonly applied to systems wherein the external phase comprises water (*i.e.*, an aqueous system). Such an external phase clearly does not meet the requirements of the instant claims for a non-polar excipient or mixture of non-polar excipients. One skilled in the art would not have any expectation of success in applying any methodology related to such aqueous systems to the instant non-polar systems and the Office has identified none.

Lafon (micelles) and Hiroshi (reverse micelles) relate to a totally different physical system, which have different properties from reversed vesicular systems (see, *e.g.* Blinkovsky, Column 2, ll. 2 – 15; and our previous discussion of the differences between micelles and vesicles). One skilled in

the art would not combine the teachings of such non-analogous art; nor would one skilled in the art have any expectation of success in applying any methodology related to micellar systems to the instant vesicular systems.

The Applicants submit that none of the cited documents cure the admitted deficiencies of the DeVringer document; they are not combinable because they relate to different systems. Therefore, the instant rejection of claims 1 – 9, 12 – 17, and 19 – 37 under 35 USC 103(a) is improper. The Applicants respectfully request reconsideration and withdrawal of the rejection.

Rejection of Claims 1 – 9, 12 – 17, and 19 – 37 under 35 USC 103(a) over EP 0 521 562 ('DeVringer') in light of US Pat. No. 5,693,516 ('Blinkovsky')

Claims 1 – 9, 12 – 17, and 19 – 37 stand rejected under 35 USC 103(a) as allegedly being obvious by combining the teachings of EP 0 521 562 ('DeVringer') and US Pat. No. 5,693,516 ('Blinkovsky'). The Applicants respectfully disagree.

We refer to our preceding discussions of DeVringer and Blinkovsky. The Applicants note that DeVringer is concerned with the production of dispersions of ***vesicles*** while Blinkovsky is concerned with the preparation of dispersions and compositions comprising ***micelles***. Therefore, one skilled in the art would not have considered combining the teachings of DeVringer with Blinkovsky because they are concerned with fundamentally different problems and are, therefore, non-analogous art. MPEP 2141.01(a).

The Applicants submit that the instant rejection of claims 1 – 9, 12 – 17, and 19 – 37 under 35 USC 103(a) is improper, and respectfully request reconsideration and withdrawal of the rejection.

CONCLUSION

Applicants respectfully submit that all requirements of patentability have been met. Allowance of the claims and passage of the case to issue are therefore respectfully solicited.

If the examiner believes a teleconference will advance prosecution, he is encouraged to contact the undersigned as indicated below.

Respectfully submitted,

Date: January 22, 2007

/Michael S. Greenfield/
Michael S. Greenfield
Registration No. 37,142

McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, IL 60606

Telephone: 312-913-0001
Facsimile: 312-913-0002

VESICLES

edited by
Morton Rosoff
Professor Emeritus
Long Island University
Brooklyn, New York

Marcel Dekker, Inc. New York • Basel • Hong Kong

Library of Congress Cataloging-in-Publication Data

Vesicles / edited by Morton Rosoff.
p. cm. — (Surfactant science series; v. 62)
Includes index.
ISBN 0-8247-9603-9 (hardcover: alk. paper)
1. Liposomes. 2. Surface chemistry. I. Rosoff, Morton. II. Series
RS201.L55V47 1996
574.87'4—dc20 96-15201
CIP

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This book is printed on acid-free paper.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Current printing (last digit):

10 9 8 7 6 5 4 3 2 1

PRINTED IN THE UNITED STATES OF AMERICA

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Formation and Structure of Reverse Vesicles

HIRONOBU KUNIEDA Department of Physical Chemistry, Yokohama National University, Yokohama, Japan

VIJAY RAJAGOPALAN Chemical Center, University of Lund, Lund, Sweden

I. INTRODUCTION

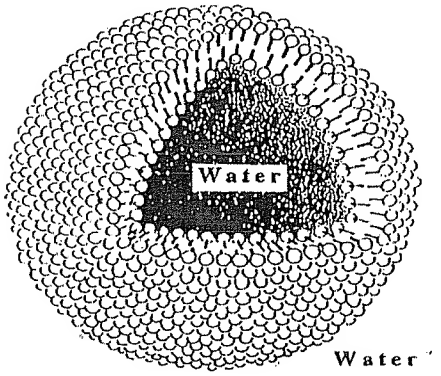
Amphiphilic compounds or, in short, amphiphiles, are compounds that possess within the same molecule two distinct groups which differ greatly in their solubility relationships [1]. The two groups of these compounds are in general termed "lyophilic" (the group having an affinity for the solvent) and "lyophobic" (the group having no affinity for the solvent). Whenever water (or oil) is used as the solvent, the two groups are referred to as hydrophilic and hydrophobic (or lipophilic and lipophobic), respectively. The physical properties of amphiphilic compounds or surfactants in aqueous solvents undergo an abrupt change over a narrow concentration range, and this change is normally accepted to be due to the formation of aggregates, and the simplest of these aggregates is often referred to as a micelle. The aggregation of amphiphilic compounds in apolar solvents is also equally possible, and the simplest or the smallest aggregate is often referred to as an inverted micelle or reverse micelle.

Surfactant molecules aggregate to form a large variety of diverse structures such as normal and reverse micelles, bilayers, etc. The site and shape of these self-organizing structures in ternary water/surfactant/oil systems are basically dictated by temperature, pressure, concentration and the interaction between the surfactant, oil and water molecules. Among the various self-organizing structures, vesicles or liposomes, which are single- or multi-layer shells of surfactants or lipids, are receiving increasing attention both because of the fundamental insight that they may provide on the self-assembly of amphiphilic substances and because of their biological significance as model membrane systems with numerous applications, notably in drug delivery [2–4].

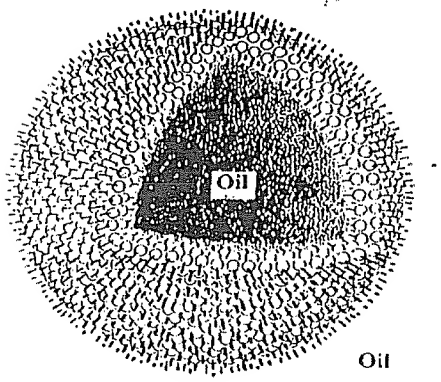
One normally observes from a phase diagram that there is symmetry of these amphiphilic molecular aggregates, such as micelles and reverse micelles, hexagonal and reverse hexagonal liquid crystals, etc. All of these have been quite extensively studied by various authors using various experimental techniques. However, until recently, not much attention has been paid to the counter structure of lamellar liquid crystals or vesicles.

It was recently demonstrated that vesicles can also be produced in nonpolar media [5–11]; and these were consequently termed reverse vesicles. Reverse vesicles consist of closed bimolecular layers with both the inside and outside being nonpolar liquid and are formed from a dispersion of a lamellar liquid crystalline phase in a nonpolar liquid. A schematic illustration of both normal and reverse vesicles is shown in Fig. 1.

Both normal as well as reverse vesicles offer a lot of scope and are very good candidates with a lot of potential for extensive use in industrial and pharmaceutical applications which range from basic membrane research to bioconversion of water-insoluble compounds in fine organic synthesis. The fact that Ferrer and Carmona [12] have already investigated the reactivity of enzyme in a reverse vesi-



(a) *Normal Vesicle*



(b) *Reverse Vesicle*

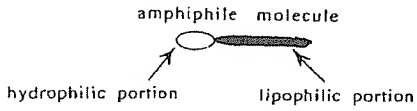


FIG. 1 Schematic models of normal and reverse vesicles. (From Ref. 9.)

cle system and shown quite conclusively that this type of aggregate protects the enzyme against denaturation, which is normally produced by the surrounding organic solvent, bears full testimony to the potential that this aggregate has to offer.

In the first section, we will describe the relationship between surfactant phase behavior and self-organizing structures. In the second section, we will discuss the structure of reverse vesicles. In the third section, we shall focus our attention on the reverse vesicles formed in the sucrose monoalkanoate system and in the fourth section we will look at the various methods to produce reverse vesicles, such as spontaneous formation of reverse vesicles, and finally in the fifth we will give a summary of the entire text.

II. SURFACTANT PHASE BEHAVIOR AND SELF-ORGANIZING STRUCTURES

A. Symmetry of Phase Behavior

In the case of nonionic surfactant systems the intermolecular interactions vary with temperature. At lower temperatures the nonionic surfactant is hydrophilic and forms normal micelles; on the other hand they are lipophilic at higher temperatures and they form reverse micelles at higher temperatures in ternary systems. At an intermediate temperature called the HLB (hydrophile-lipophile balance) temperature, the hydrophile-lipophile interactions just balance each other and an isotropic surfactant phase (microemulsion) coexists with both excess oil and water phases [13–15]. At the HLB temperature, the average curvature at the oil-surfactant-water interface is zero and the microemulsions have a bicontinuous structure [16, 17]. Thus, we see that temperature is an important parameter in nonionic surfactant systems.

The phase diagram of a ternary system at constant surfactant concentration reveals the relationship between surfactant phase behavior and types of aggregates and dispersions (emulsions) clearly and is shown in Fig. 2.

A detailed phase diagram elucidating the symmetry and the effect of temperature on the water /$R_{12}EO_5$/ tetradecane system has already been reported by H. Kunieda and K. Shinoda [18].

At lower temperatures, the surfactant forms aqueous micelles, and beyond the solubilization limit the aqueous micellar solution phase coexists with excess oil phase. In the two-phase region, O/W-type emulsions are formed. In an oil-rich region, the volume fraction of internal oil phase exceeds the critical volume fraction of close-packed spheres and the O/W emulsions become viscous and translucent. Therefore, these highly concentrated emulsions are often referred to as high internal phase ratio emulsions (HIPREs) or, in short, gel emulsions. The existance, stability and structure of these gel emulsions have been well studied and characterized [19–25]. Recent electron spin resonance (ESR) studies [26, 27] have

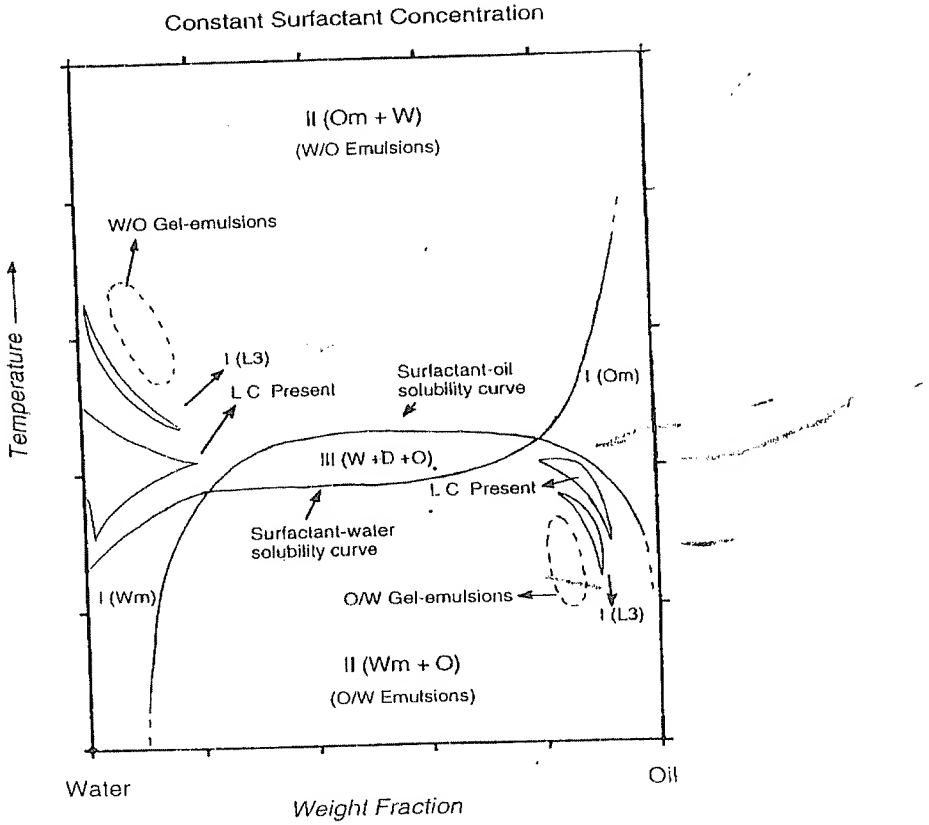


FIG. 2 Effect of temperature on the surfactant phase behavior and types of self-organizing structures in a water/nonionic surfactant/oil system.

helped us to better understand the structure and factors affecting the stability of these systems.

In nonionic surfactant systems as the temperature increases, the aggregation number also increases, and hence the solubilization of oil increases, and eventually, large oil-swollen micelles get separated as a isotropic surfactant phase or middle-phase microemulsion (D phase), which coexists with excess water and oil phases at the HLB temperature.

Above the HLB temperature, surfactant is oil-soluble and forms reverse micelles which solubilize water. Beyond the solubilization limit, reverse micellar

solution phase coexists with excess water phase, and W/O-type emulsions are formed in the two-phase region. At the same time W/O-type emulsion or gel-like highly concentrated emulsions can also be formed in the water-rich regions at higher temperatures.

Around the HLB temperature, lamellar liquid crystal forms in both water- and oil-rich regions as shown in Fig. 2. These liquid crystals are continuously connected in a concentrated region because both the water and oil content in liquid crystals decrease. When the liquid crystals swell due to the addition of large amounts of water or oil and the interlayer spacings become large, the surfactant bilayers become flexible and undulate [28]. Olsson et al., using NMR [16], have shown that the D phase has a layered structure with alternating water and hydrocarbon layers separated by monolayers of surfactant. The structure is highly dynamic and flexible, and the surfactant layers dissociate and reassociate on a short time scale. Thus the surfactant phase can be described as a dynamic and disordered, highly swelled lamellar phase which solubilizes a large amount of water and oil.

The complete phase diagram around the HLB temperature in a water/$R_{12}EO_4$/dodecane system is shown in Fig. 3. The HLB temperature or the median temperature of the main three-phase body of this system is around 25°C. Lamellar liquid crystals also exist in the water-$R_{12}EO_4$ binary system [29]. Above the cloud point temperature of 4°C [30] the LC phase intrudes into the cloud point curve and coexists with the excess water in the water-surfactant axis. An isolated isotropic surfactant phase (D phase) coexists with both excess oil and water phases and forms a three-phase triangle. The D phase is not symmetrically placed in the center of the phase diagram, because the median temperature of the three-phase triangle is slightly below 25°C.

As the lateral interactions between the surfactant molecules are quite small for shorter-chain surfactants the isotropic phase region in such systems gets expanded and the D phase connects the water and oil apexes [15]. However, in the water /$R_{12}EO_4$/dodecane system the D phase engages in two types of three-phase triangles, including the lamellar LC, which intrudes into the water- and oil-rich regions.

The surfactant readily dissolves in oil to form isotropic solutions and no LC is present, but on the addition of a small amount of water these isotropic solutions split into a lamellar liquid crystalline phase which is in equilibrium with an excess oil phase [18, 31]. Unlike most lamellar liquid crystals, the $R_{12}EO_4$ system solubilizes large amounts of hydrocarbon within the single-phase region, as shown in Fig. 3. The LC phase being quite broad, the structure of the LC phase in the water-rich region consists of bilayers separated by large amounts of water and on the oil-rich side the bilayers are separated by a thick oil layer. SAXS data has confirmed that the addition of oil increases the interlayer spacing in the adjacent water-EO bilayer regions as shown in Figs. 4 and 5 [32, 33]. This reversed bilayer system parallels normal bilayer systems in which addition of water increases the spacing between the hydrocarbon bilayers. Consequently the orientations of

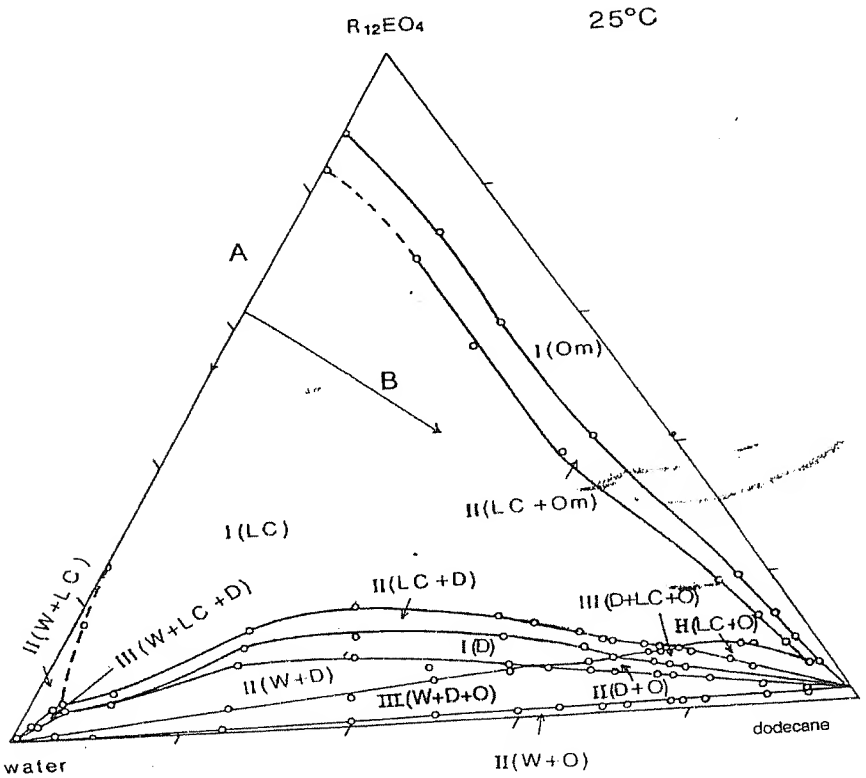


FIG. 3 Phase diagram for a water/$R_{12}EO_4$/dodecane system at 25°C: LC, lamellar liquid crystal; D, isotropic surfactant phase; I, II, and III, one-, two-, and three-phase regions, respectively. W and O are excess water and oil phases. Om is the reversed micellar solution phase. (From Ref. 10.)

the surfactant molecules are opposite. In the water-rich region they are of the normal LC type, whereas in the oil-rich region they are of the reverse LC type.

The fact that the LC phase swells by solubilizing either large amounts of water or oil can be confirmed by SAXS, in which one essentially measures the separation of the neighboring bilayers. Figure 4 shows the interlayer spacing of a lamellar LC phase in a binary, water-$R_{12}EO_4$ system as a function of the reciprocal of the surfactant volume fraction with the assumption that the partial molar volume of each of the constituents is the same as that in the pure state. If one considers the interlayer spacing d to be the sum of the thickness of the bimolecular layer of the

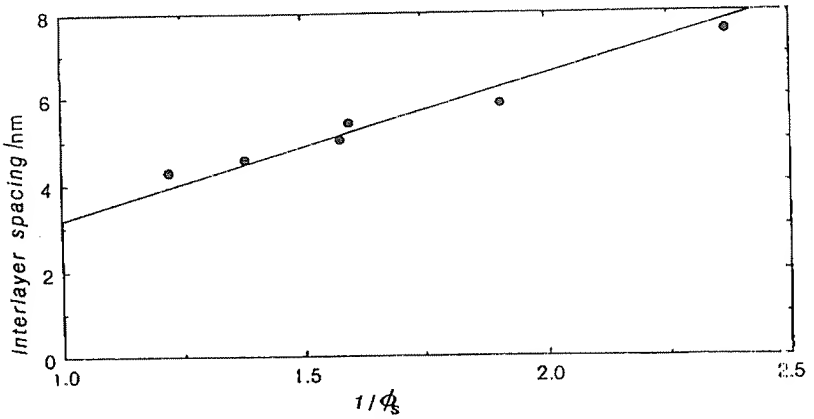


FIG. 4 Interlayer spacing of lamellar liquid crystalline phase in a binary system of water-$R_{12}EO_4$ as a function of surfactant concentration. (From Ref. 10.)

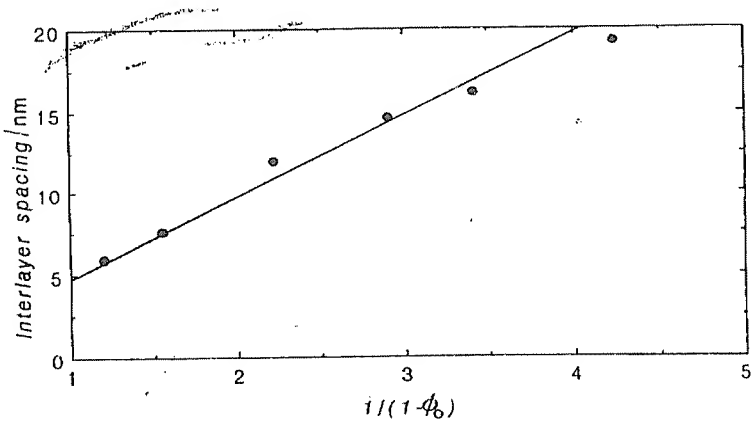


FIG. 5 Interlayer spacing of lamellar liquid crystalline phase along the path A shown in Fig. 3 as a function of excess oil phase content. (From Ref. 10.)

surfactant, d_s, plus that of water, d_w, and if d_s is a constant for the binary system and only d_w increases with increasing water content, then a plot of the reciprocal of volume fraction to the interlayer spacing should be a straight line with the intercept being equal to d_s. Figures 4 and 5 show that the swelling of the bilayer on the addition of water is relatively small compared to the amount of swelling ob-

served with oil, where the addition of oil was along path B of Fig. 3. It is known that the cross-sectional area per surfactant molecule in the bilayers does not significantly change on the addition of hydrocarbon to the lamellar LC [34]. As d_w and d_s are constant along path B,

$$d = \frac{d_w + d_s}{\phi_s + \phi_w} \tag{1}$$

where ϕ_s and ϕ_w are the volume fractions of surfactant and water, respectively. Thus Figs. 4 and 5 confirm that the liquid crystals solubilize large amounts of oil or water by increasing the nonpolar to polar interlayer spacing.

Reverse vesicles are formed in the two-phase system containing liquid crystals and excess oil, as can be seen from Fig. 3. Like normal vesicles, reverse vesicles also coalesce and revert to lamellar liquid crystalline phase behavior over a period of hours to days.

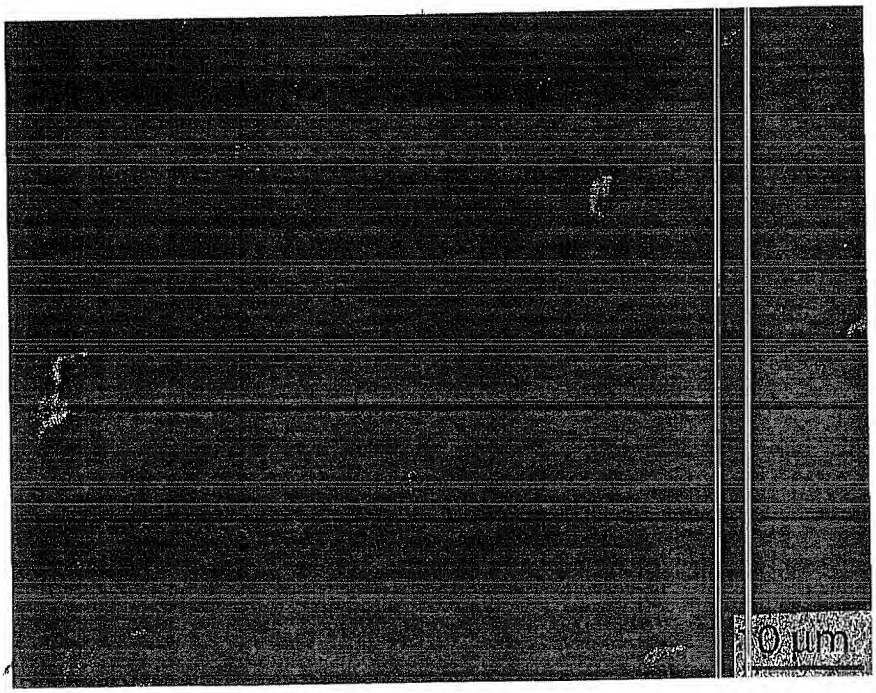
We also notice from Fig. 3 that the LC in an oil-rich region is extended toward lower temperatures from the HLB temperature, whereas in a water-rich region it is extended to higher temperatures. This means that in order to form reverse vesicles, the surfactant should be hydrophilic, whereas relatively lipophilic surfactants can be used for the formation of normal vesicles.

If one uses a hydrophilic surfactant it is seen that reverse vesicles can also be formed at temperatures below the HLB temperature in nonionic surfactant systems [8]. Thus, reverse vesicles can be formed at temperatures at and below the characteristic HLB temperature of the system. In other words, the hydrophile-lipophile property of the surfactant should be balanced or hydrophilic for the formation of reverse vesicles.

Judging from the symmetry patterns in phase diagrams, although it is reasonable for one to expect the existence of reverse vesicles, normal vesicles can also be formed using lipophilic surfactants whose HLB temperature is lower than experimental temperature, as can be seen from Fig. 3.

B. Various Reverse Vesicular Systems

The first evidence of formation of reverse vesicles was with nonionic surfactants, but the first evidence with ionic surfactants was in the AOT-SDS/decane/water system [11]. A considerable amount of water was solubilized in the AOT nonaqeuous solution. When AOT is partially replaced with a more hydrophilic surfactant like SDS, the lamellar liquid crystalline phase gets separated from the oil phase. As the SDS content is increased, the amount of water required to form the lamellar liquid crystal decreases. This lamellar liquid crystal, when shaken, gives rise to reverse vesicles. Thus the combination of a less hydrophilic oil-soluble ionic surfactant and a hydrophilic surfactant is important for the formation of reverse vesicles. But the most important factor seems to be the addition of a small amount of water in order to adjust the interactions of the hydrophilic parts.

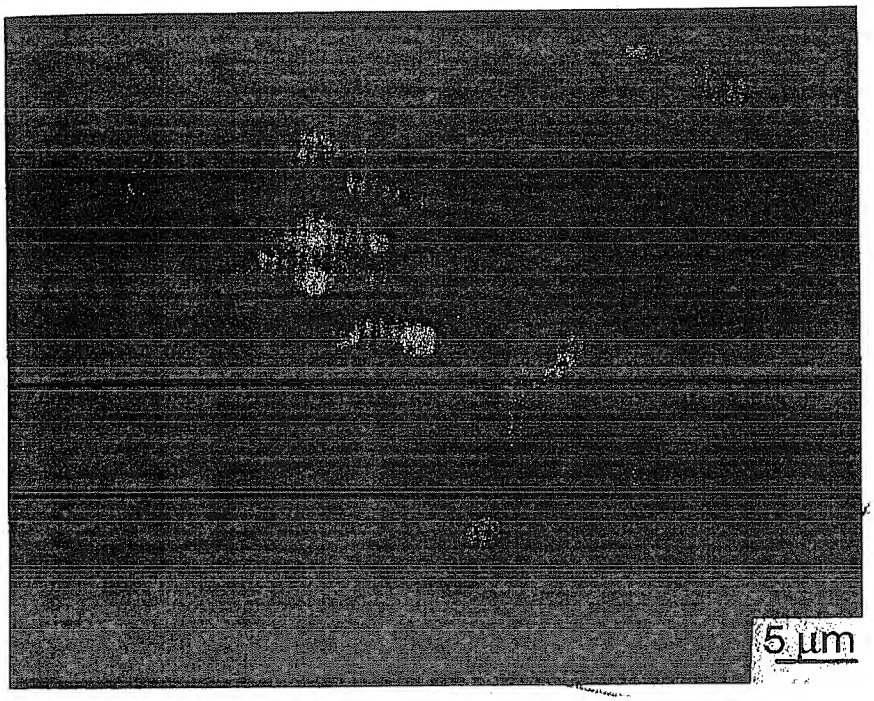


(a)

FIG. 6 (a) Reverse vesicles formed by mixing LC phase and decane (ultrasonication for 20 min). DKE 1.2 wt%, $R_{16}EO_6$ 1.8 wt%, and decane 97 wt%. (From Ref. 35.) (b) Fluorescence microphotograph for reverse vesicles at 30°C. DKE 1.2 wt%, $R_{16}EO_6$ 1.8 wt %, and decane (containing 0.5 wt% pyrene) 97 wt%. (From Ref. 35.)

Reverse vesicles can also be formed in a mixture of ionic and nonionic surfactants [6]. Reverse vesicles are formed in a mixture of nonionic (monooleoyldiglycerol; DGMO) and ionic (sodium dodecyl sulfate; SDS) surfactants.

In order to make use of reverse vesicles as advanced materials for medicines, pesticides, foods, cosmetics, etc., it is of utmost importance to use biocompatible surfactants such as phospholipids, amino acid derivatives, etc. If extremely hydrophilic surfactants are used, the surfactants would be incompatible with oil, and a lamellar liquid crystal which swells, encasing a large amount of oil, cannot be obtained. In such a case, the dispersibility of the liquid crystal is too poor to form reverse vesicles. Therefore the right combination of hydrophilic and lipophilic surfactants is very important for the formation of reverse vesicles. Investigations have



(b)

been done [7] for four combinations: (i) lecithin-N^{α}-lauroyl arginine methylester hydrochloride (LAM), (ii) lecithin-lysolecithin, (iii) glycerol monolaurylether-LAM and (iv) hexanol-lysolecithin, in which the former amphiphiles are lipophilic and are important biocompatible amphiphiles.

III. STRUCTURE OF REVERSE VESICLES

A. Optical Microscopy

Large-sized (10 to 20 µm) reverse vesicles can be detected by optical microscopy. As the difference in refractive indices between reverse vesicles and continuous media is normally quite small in some systems, a differential interference phase-contrast microscope equipped with an image processor (VEM, video-enhanced microscopy) is very useful to detect reverse vesicles [5]. Figure 6a shows VEM pictures of large multilamellar reverse vesicles, and Fig. 6b shows the fluorescence

microphotograph for reverse vesicles at 30°C. If the reverse vesicular system contains a small amount of water, then the reverse vesicular structure can be also observed by fluorescence microscopy by making use of water-soluble dyes such as calcein, etc. Since water is concentrated in a bilayer, a bright ring is observed in the case of large vesicles.

B. Freeze-Fractured Transmission Electron Microscopy

When one wants to observe the detail structure of reverse vesicles in the submicron range, then one makes use of electron microscopy. This technique is quite useful to confirm the unilamellar or multilamellar nature of the reverse vesicle.

Figure 7 shows the TEM photograph of the reverse vesicles formed in the water/sucrose monoalkanoate/hexanol/decane system for the following composition: sucrose monoalkanoate, 4.5 wt%; water, 0.6 wt%; hexanol, 9.1 wt%; and decane, 85.8 wt%. The vesicle at the lower left was cut at the upper part, and the oil phase slightly appears at the top. The multilayers were cut irregularly. For the one to the lower right, a cut was made at the lower part of the reverse vesicle and the inside oil phase was removed. Several layers can be seen at the peripheral part; hence this is considered to be a multilamellar.

The width of each layer is about 10 nm, corresponding to the oil-swollen lamellar liquid crystals [10]. The SAXS data show that the interlayer spacing is 10.9 nm at this composition. Therefore, the layer structures of reverse vesicles correspond to reversed bilayers.

On the other hand, the molar ratio of water to sucrose ester is small, only about 4.5 in the vesicular system. It is therefore considered that the hydrophilic part of the bimolecular layers is not separated by water, whereas the lipophilic parts are largely separated by decane. Accordingly, the bimolecular layers in the vesicles are of the reverse type in which the hydrophilic parts are oriented toward the inside.

IV. REVERSE VESICLES IN A SUCROSE MONOALKANOATE SYSTEM

Very stable reverse vesicles are formed in a water/sucrose monoalkanoate/hexanol/decane or a sucrose monoalkanoate/hexaethyleneglycol hexadecyl ether ($R_{16}EO_6$)/hydrocarbon system [11,35]. Sucrose monoalkanoate is nonionic, hydrophilic, has very low monodispersity in the oil phase, does aggregate very easity and, above all, is biocompatible. Even though it is nonionic no clouding is observed up to 100°C. Sucrose monoalkanoate is not soluble in hydrocarbons and precipitates as a hydrated solid at lower temperatures, but if it is mixed with a small amount of water a lamellar liquid crystal is formed in decane, and on the addition of hexanol the lamellar liquid crystal swells a large amount of decane. It was seen

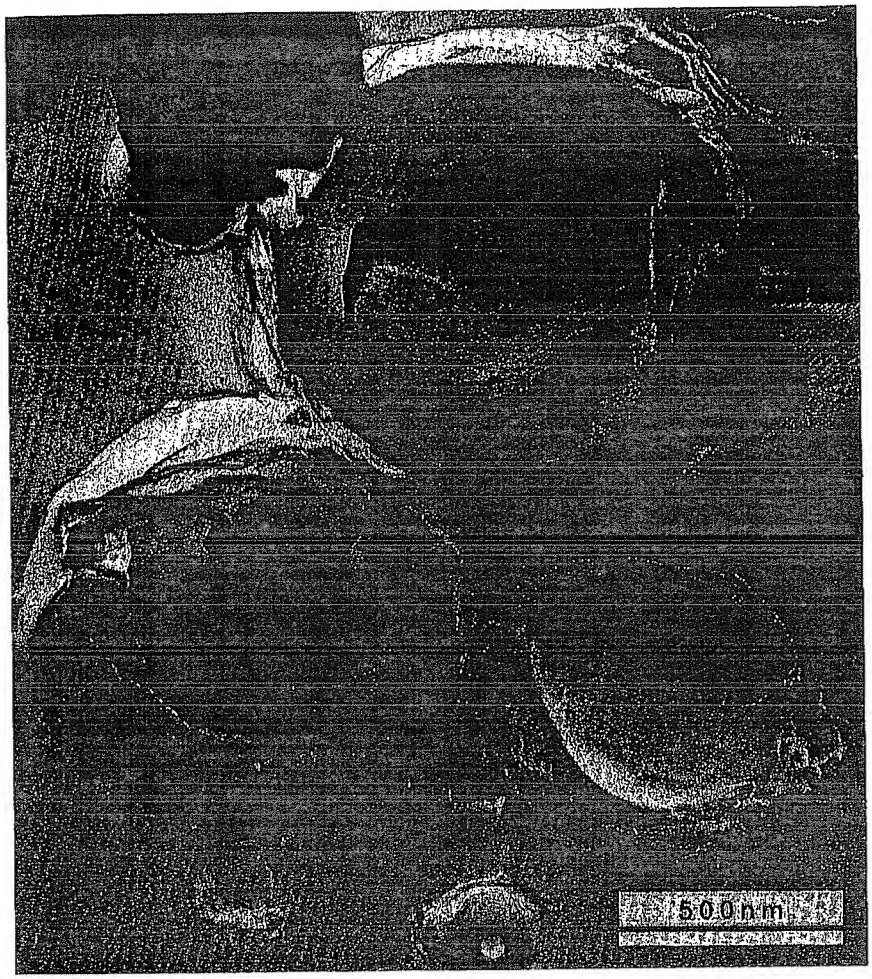


FIG. 7 Cryo TEM picture of large and small reverse vesicles. (From Ref. 9.)

that very stable reverse vesicles of sucrose monoalkanoate can be formed in a decane + hexanol system in the presence of small amounts of water.

The first observation of spontaneous formation of reverse vesicles without addition of water was observed in the system composed of a sucrose monoalkanoate (DKE), hexaethyleneglycol hexadecyl ether ($R_{16}EO_6$) and decane.

In the following section, we describe the phase behavior and the lamellar liquid crystal phase of the DKE/$R_{16}EO_6$/decane system.

A. The Solid to Lamellar Phase Transition

Phase transition from a gel (solid) state to a liquid crystalline state occurs in normal vesicular systems. $R_{16}EO_6$ is in a solid state in decane at lower temperature. Therefore, there is a solid-liquid crystal transition in a DKE/$R_{16}EO_6$/decane system and is illustrated in Fig. 8.

A lamellar liquid crystalline phase is not formed in a binary $R_{16}EO_6$-decane system, in which the phase transition from a solid state to an isotropic solution occurs at 25°C. The transition enthalpy is estimated to be about 50 kJ mol^{-1}, which corresponds to the value for melting of a hydrocarbon [36]. With the increase in DKE content, the phase transition temperature decreases and a solid-to-liquid crystal transition is observed above 27 wt% DKE, in total surfactants. This

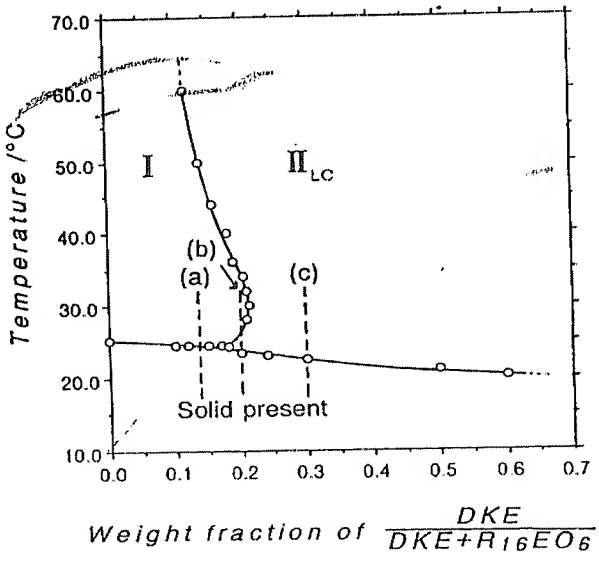


FIG. 8 The phase diagram of a DKE/$R_{16}EO_6$/decane system, as a function of temperature, and the mixing ratio of amphiphiles. The concentration of DKE-$R_{16}EO_6$ mixture is fixed at 5 wt% in system. The "solid present" means the region containing a solid phase. LC means lamellar liquid crystalline phase. II_{LC}: a two-phase region consisting of LC and isotropic phases. I is a single-isotropic-phase region. (From Ref. 35.)

phase transition was observed using DSC, and some typical DSC traces are as shown in Fig. 9.

The DSC traces (a), (b), and (c) correspond to the lines (a), (b), and (c) of the phase diagram (Fig. 8). There is only one endothermic peak in (a), whereas two peaks are observed in (b). The peak in (a) corresponds to the phase transition between the solid state and the isotropic phase. On the other hand, first and second peaks in (b) correspond to the phase transitions between the solid state and the liquid crystalline state, and the liquid crystalline state and the isotropic state, respectively. The peak in (c) is the same as the first peak in (b).

The gradual decrease in the phase transition *curve* indicates that $R_{16}EO_6$ and DKE form mixed aggregates in the isotropic liquid and liquid crystalline states.

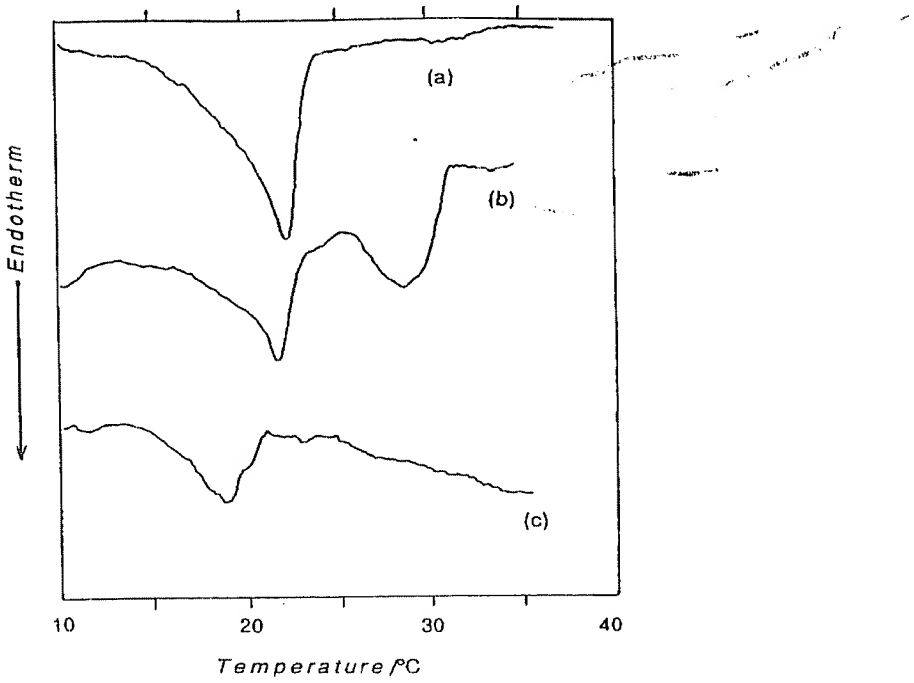


FIG. 9 DSC thermograms of phase transitions in a DKE/$R_{16}EO_6$/decane system. The compositions correspond to the broken lines in Fig. 8. Each thermogram exhibits (from Ref. 35.) (a) solid to isotropic phase, (b) solid to liquid crystalline phase to isotropic phase, (c) solid to isotropic phase.

B. Phase Diagram of the DKE/$R_{16}EO_6$/Decane System

In the presence of decane, the mixture of DKE and $R_{16}EO_6$ is in a liquid state at 30°C for a wide range of compositions. The phase diagrams for the DKE/$R_{16}EO_6$/decane and DKE/$R_{16}EO_6$/heptane systems at 30°C are as shown in Figs. 10 and 11.

The two systems show a very similar phase behavior except for the isotropic single-phase areas. A single isotropic solution phase (I) exists in the $R_{16}EO_6$-rich region, whereas a liquid crystalline phase is present in the DKE-rich region.

The two phases are separated by a two-phase region (II_{LC}). The tie lines were examined in the heptane system and were found to be approximately parallel to the DKE-heptane binary axis as shown. In general, the lamellar phase is in equilibrium with a solution of $R_{16}EO_6$ in oil which is consistent with the high oil solubility of $R_{16}EO_6$.

We also note the particular shape of the phase boundary of the isotropic liquid phase in the vicinity of the oil corner. The oil-rich solution must exceed a certain threshold concentration of $R_{16}EO_6$ before it can solubilize DKE.

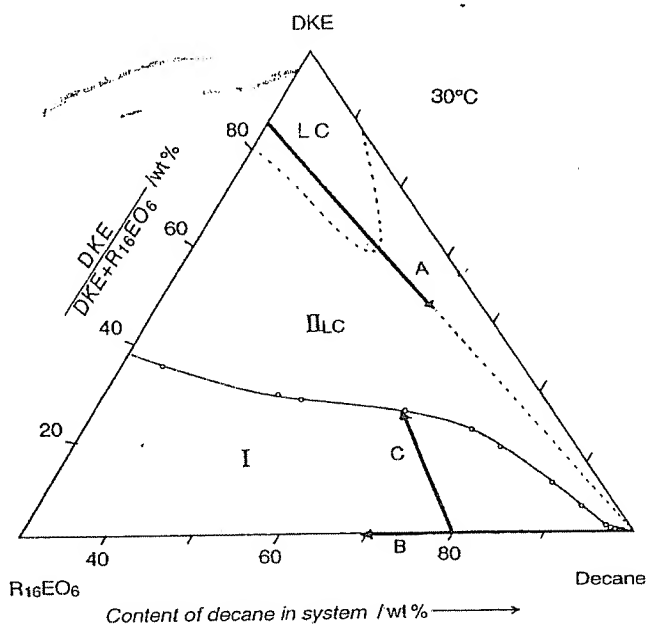


FIG. 10 Partial phase diagram in a DKE/$R_{16}EO_6$/decane system at 30°C. LC means a single lamellar liquid crystalline phase. II_{LC}: two-phase region consisting of LC and isotropic phases. I is a single-isotropic-phase region. (From Ref. 35.)

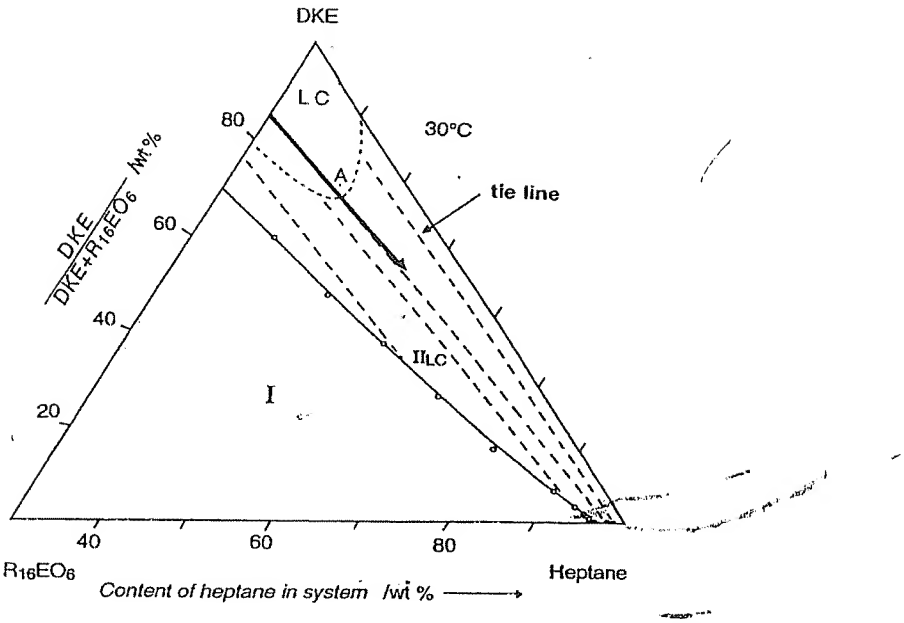


FIG. 11 Partial phase diagram in a DKE/$R_{16}EO_6$/heptane system at 30°C. The tie lines between LC and isotropic phase were determined by means of HPLC. (From Ref. 35.)

C. The Lamellar Phase

Samples in the liquid crystalline phase display a typical lamellar phase texture when viewed with the help of a polarizing microscope. The lamellar structure was further confirmed by small-angle x-ray scattering (SAXS), which shows, in addition to the first order, also a second-order peak in the diffraction pattern with the relative peak positions in the ratio of 1:2 which is characteristic of a lamellar structure.

SAXS was used to investigate the swelling of the lamellar phase with oil along a dilution line defined by a constant DKW-to-$R_{16}EO_6$ weight ratio of 4 (shown as line A in the phase diagrams, Figs. 10 and 11). The repeat distance in a lamellar structure is given by $d = d_s + d_o = d_s/\Phi_s$, where d_s and d_o are the surfactant bilayer and thickness of the oil layer respectively, and Φ_s is the surfactant volume fraction. Pictorial representation of d_s and d_o are shown in Fig. 12. Expressing Φ_s in terms of weight fractions and densities, we have

$$d = d_s\left(\frac{W_o\rho_s}{1 + W_s\rho_o}\right) \tag{2}$$

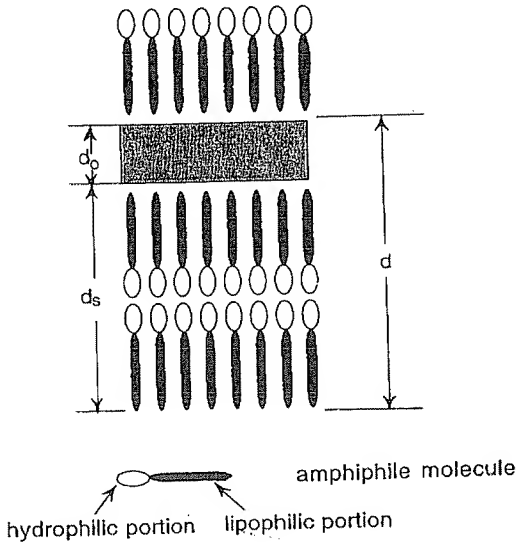


FIG. 12 Schematic representation of lamellar liquid crystalline phase swelling hydrocarbon. d_s: interlayer spacing of bimolecular layer. d_o: interlayer spacing of hydrocarbon. (From Ref. 35.)

where ρ_s and ρ_o are the densities of surfactant and oil, respectively. A plot between the smectic repeat distance, d, calculated from the scattering angle at the first-order Bragg singularity, and the oil-to-surfactant weight ratio, W_o/W_s is shown in Fig. 13, where W_s is the total weight fraction of the surfactant mixture. We see that d varies linearly with W_s/W_o, which is indicative of a one-dimensional swelling and hence from the extrapolation of the data to $W_s/W_o = 0$, we obtain $d_s = 4.4$ nm (decane) and 4.05 nm (heptane) as the bilayer thickness. We note that the Bragg singularity in the diffraction pattern, in principle, reports on the bilayer area per unit volume, and the bilayer thickness, d_s, in Eq. (2) is given by the average volume-to-area ratio of the bilayer molecules. In our case we identify the bilayer units as a reverse bilayer, and d_s is expected to be smaller than twice the extended length of the surfactant molecules. From the CPK space-filling model, the extended length of a sucrose monostearate molecule can be estimated to be about 3.4 nm. As the DKE used is a mixture of homologs and the average carbon number of hydrocarbon chain is about 17, therefore, the extended length of the DKE molecule is estimated to 3.15 nm. The fact that d_s is considerably smaller than this value signifies the liquid-like character of the reverse bilayer membrane. Moreover, since oil molecules penetrate bilayers, the effective

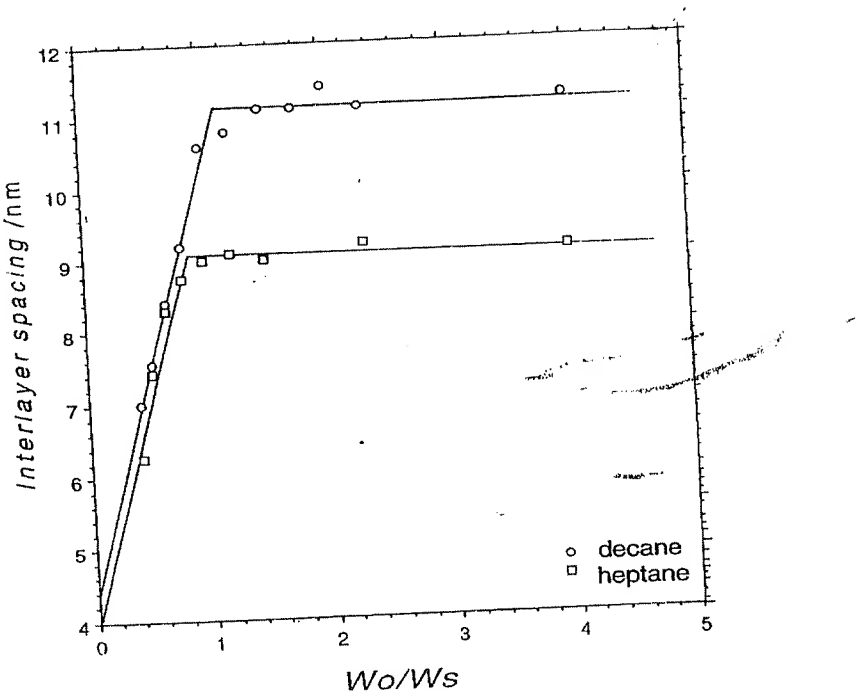


FIG. 13 Interlayer spacings, d, in the lamellar phase as a function of the decane-to-surfactant (○) or heptane-to-surfactant (□) weight ratio, measured by SAXS along the dilution path A, shown in Figs. 10 and 11. (From Ref. 35.)

bilayer thickness observed by SAXS is bound to be smaller than the actual bilayer thickness because the penetrated oil acts as the background in our measurements. The observed difference in the d_s spacings can be attributed to the fact that, as the heptane molecule is smaller than decane, the heptane molecule would penetrate the bilayer to a greater extent in comparison with decane. Hence, the observed difference in d_s spacings for heptane is, as expected, lower than that observed for decane.

The lamellar phase can swell with oil up to a repeat distance of about 11.2 nm for decane and 9.1 nm for heptane. At contact, we expect a repulsive steric-type force, arising from an unfavorable interpenetration of hydrocarbon chains. The long-range repulsion responsible for the swelling of the bilayers away from contact must, since the bilayers are electrically neutral, be due to a Helfrich undulation force [37]. The swelling, however, is rather limited if we, for example, compare with the (normal) bilayers of $R_{12}EO_5$ where a lamellar phase swells with

water to repeat distances of the order of several hundred nanometers [36]. Some of our recent SAXS studies in the water/DKE/$R_{16}EO_6$/decane system shows that the addition of water seems to promote the squeezing out of the oil from the bilayer, thereby resulting in $d_o \approx 0$. This seems to be consistent with our observation that, as the water content in the system increases, the reverse vesicles initially formed become less stable. Thus, as one increases the water content we only get a lamellar liquid crystalline phase dispersed in the oil phase and not a reverse vesicle. This point seems to hold the clue to the explanation of the stability of these systems.

D. The Isotropic Solution Phase

The isotropic liquid phase includes the binary $R_{16}EO_6$-decane axis and extends into the ternary system upon addition of sucrose alkanoate. Nonionic surfactants of the ethylene oxide type are in general completely miscible with hydrocarbons, above their melting points, but do not self-associate into micelles in the absence of water [38], a fact that can be seen from the absence of liquid crystalline phases at higher surfactant concentrations and has also been demonstrated by NMR relaxation and self-diffusion studies [28]. In the ternary system including sucrose alkanoate, we find a lamellar liquid crystalline phase for compositions rich in sucrose alkanoate (Figs. 10 and 11). Hence, one would expect in the liquid isotropic phase a progression from an unstructured to a structured (reverse micellar) solution upon increasing the sucrose alkanoate-to-$R_{16}EO_6$ ratio.

The microstructure of the liquid isotropic phase was studied by measuring the molecular self-diffusion coefficient of $R_{16}EO_6$ (at 30°C) by the FT-PGSE NMR technique. In the NMR study, deuterated decane ($C_{10}D_{22}$) was used in order to properly record the resonances from the $R_{16}EO_6$ amphiphile. The measurements were performed on a dilution line on the binary $R_{16}EO_6$-decane axis (dilution line B of Fig. 10) and on a line of increasing sucrose alkanoate concentration in the ternary system for a fixed $R_{16}EO_6$-to-decane ratio, 1/4 by weight (line C in Fig. 10). The results of the self-diffusion measurements are as shown in Fig. 14, where the self-diffusion coefficient of $R_{16}EO_6$ is plotted as a function of the total amphiphile concentration. Note that below 20 wt%, the data refer only to the binary $R_{16}EO_6$-decane axis. From 20 wt%, the total amphiphile concentration is increased further either by adding additional $R_{16}EO_6$ (open symbols) or by adding DKE (filled symbols).

On the binary $R_{16}EO_6$-decane axis, the self-diffusion coefficient of $R_{16}EO_6$ decreases smoothly with increasing amphiphile concentration. The self-diffusion coefficients are relatively high, which is indicative of the absence of micelle formation, and the decrease with increasing concentration can be understood as a viscosity effect, which one encounters when one mixes a more viscous liquid ($R_{16}EO_6$) with a less viscous one (decane). This behavior is analogous to what one

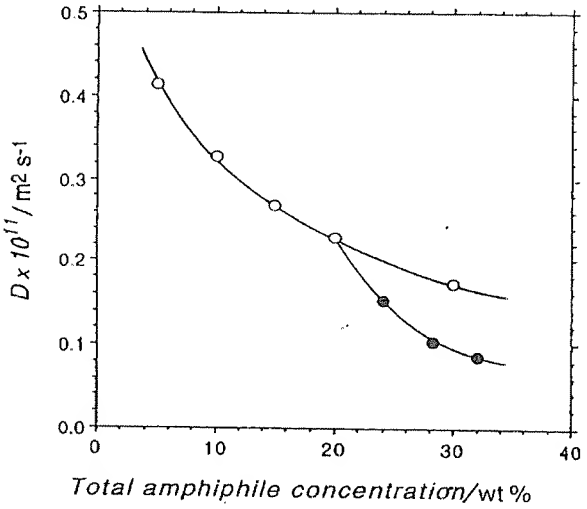


FIG. 14 Self-diffusion coefficients of $R_{16}EO_6$ as a function of the total surfactant concentration at 30°C measured by the FTPGSE NMR technique, along the paths B and C in the phase diagram (Fig. 10). The open circles correspond to measurements on the binary $R_{16}EO_6$-decane axis (path B), while filled circles refer to the three-component mixture (path C). The measurements were performed using perdeuterated decane ($C_{10}D_{22}$). (From Ref. 35.)

observes when mixing glycerol (more viscous) and water (less viscous). When adding DKE to the 20 wt% solution of $R_{16}EO_6$ in decane, the self-diffusion coefficient of $R_{16}EO_6$ decreases significantly more strongly than when adding additional $R_{16}EO_6$, demonstrating that self-association involving also $R_{16}EO_6$ molecules, occurs. Presumably, the sucrose alkanoate amphiphile occurs mainly in the aggregates, which are in a medium which is a $R_{16}EO_6$-decane mixture. The $R_{16}EO_6$ amphiphile, on the other hand, is clearly distributed between the aggregates and the continuous solvent. This distribution is shifted from the solvent toward the aggregates when one increases the DKE concentration.

Additional support for aggregation occurring upon the addition of DKE is obtained from the observed broadening of the ethylene oxide 1_{HNMR} resonances.

V. METHODS TO PRODUCE REVERSE VESICLES

In this section, typical methods used for the preparation of reverse vesicles are described.

A. Preparation of Reverse Vesicles

To prepare multilayer reverse vesicles, add the surfactants, water and oil in the right concentration in a sealed tube and slightly warm the system to a temperature above the solid-liquid phase transition temperature (this temperature is a characteristic of each system, and in order to determine this temperature it is advisable to make a phase diagram of the system and then determine the transition temperature of the system. Then, keep the tube in the vortex mixer until the system completely dissolves, put this in the sonicator (bath type) for about 20 min and you will have a slightly turbid solution that will contain reverse vesicles which are multilayered. Unilayer reverse vesicles can be prepared in the same manner as that of the multilayer type, but instead of using a bath-type sonicator, if one makes use of a high-powered probe-type sonicator, then a clear bluish solution containing unilayered reverse vesicles will be obtained.

B. Spontaneous Formation of Reverse Vesicles

Spontaneous formation of reverse vesicles is also possible and the aqueous vesicle systems provide a suitable reference. With respect to the possibility of a spontaneous formation on dilution of a solution, the aqueous lecithin-bile salt system provides a good example. This system is composed of one very insoluble lipid (lecithin), with a spontaneous curvature such that a closely planar aggregate is formed, and one lipid with a high monomeric solubility and which prefers to form closed aggregates with a curvature toward the nonpolar part (normal micelles). On dilution of a mixed micellar solution, the micelles are enriched in the less soluble component leading to a decrease of the spontaneous curvature and consequently to a micellar growth and subsequently to the entrance of the lamellar-isotropic solution in to a two-phase region. This leads to a larger possibility of stabilizing vesicles and to a spontaneous transition from micelles to (metastable) vesicles [39–42].

To imitate these conditions in a hydrocarbon system, we have chosen a mixture of two different surfactants, one of which is highly insoluble in a hydrocarbon and has a strong tendency to form a lamellar liquid crystalline phase, and one which has a high monomeric solubility and has no tendency to form larger aggregates. For the former, we chose a carbohydrate-based surfactant, a sucrose alkanoate, since these head groups are well known to be strongly oleophobic, and for the latter an ethoxylated long-chain alcohol, since the olio(ethylene oxide) surfactants are well known to have a high solubility in different nonpolar liquids.

Reverse vesicles are formed in the II_{LC} region and there are two methods to form reverse vesicles. First, they can be produced by dispersing a lamellar liquid crystal in decane. The two-phase system was shaken by a vortex mixer and sonicated.

Reverse vesicles can be formed by diluting the isotropic solution with decane. We see that at the instant of contact between the isotropic phase and decane, fine

reverse vesicles are formed in decane. At this instant, the boundary between the two phases shows a lot of turbulence. After 15 min, the turbulence dies down and we can observe large-sized reverse vesicles or liquid crystalline phase at the boundary and fine reverse vesicles in the oil phase as shown in Fig. 15.

Judging from the tie lines and the NMR measurements, the monodisperse solubility of $R_{16}EO_6$ is much higher than that for DKE, and there exist reverse micelles in the original isotropic solution. By diluting with decane, $R_{16}EO_6$ is transferred to the continuous oil medium, and the reverse micelles become DKE-rich and the aggregation number increases. Ultimately, huge aggregates are separated from the oil phase as reverse vesicles.

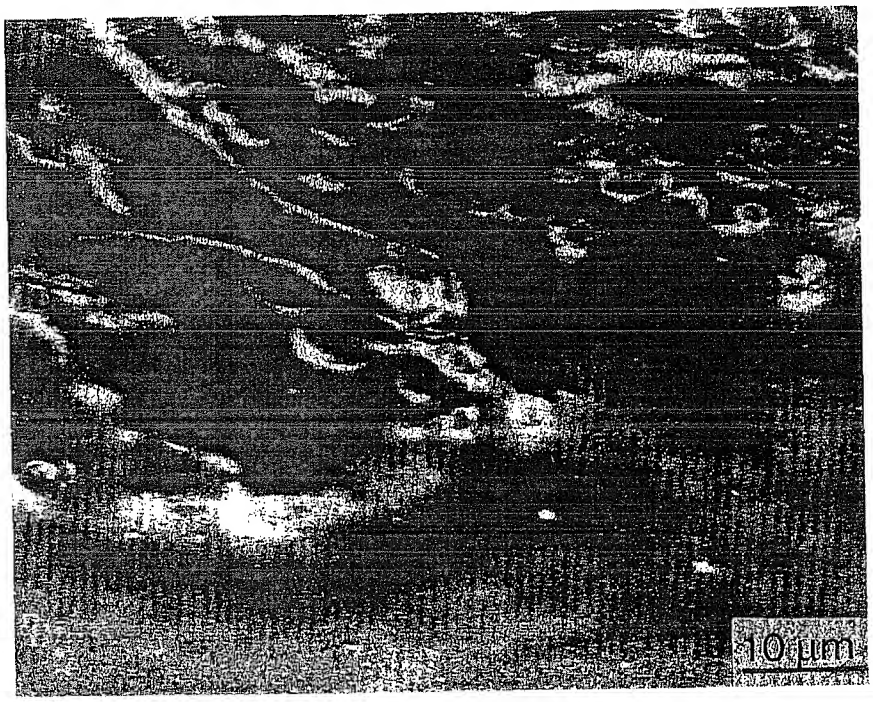


FIG. 15 Spontaneous formation of reverse vesicles by diluting isotropic solution with decane. When an surfactant isotropic solution (8 wt% DKE, 12 wt% $R_{16}EO_6$, 80 wt% decane) is in contact with pure decane, small reverse vesicles are spontaneously formed at the boundary. After 15 min, large reverse vesicles and myelin figures were observed. (From Ref. 35.)

VI. CONCLUSIONS

There is a symmetry of the self-organizing structures. Types of emulsions and self-organizing structures are highly related to the phase behavior of surfactant in water and oil. In Sec. I, the effect of temperature and water-to-oil ratio on the phase behavior and types of self-organizing structures is described in a ternary nonionic surfactant/oil/water system. The correlation between the HLB temperature or the HLB of the surfactant and the formation of reverse vesicles has been discussed. The various reverse vesicular systems observed in various systems has also been described. Section II discusses the two main methods used for the observation of structure of these reverse vesicles, namely, optical microscopy and freeze-fractured transmission electron microscopy. Freeze-fractured transmission electron microscopy is seen to be quite useful for detecting the unilamellar or multilamellar nature of the reverse vesicle. Section III describes the reverse vesicles in a sucrose monoalkanoate system. In this section the details of the phase diagram and the various experimental methods that were used for their characterization has been discussed. Finally Sec. IV describes the methods to produce reverse vesicles and the type of reverse vesicle that one normally obtains when a certain method is used for preparation. In the same section, the spontaneous formation of reverse vesicles has also been discussed, and it has been shown that one can achieve spontaneous formation of reverse vesicles by diluting isotropic solutions with hydrocarbon.

ACKNOWLEDGMENTS

Support by the Ministry of Education, Science, and Culture of Japan (Scientific Research No. 03453005) is fully acknowledged.

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